Analysis of the Longitudinal Effects of AFM24, a CD16A/Epidermal Growth Factor Receptor-Targeting (EGFR) Bispecific Innate Cell Engager, Confirms the Mechanism of Action and Supports the Rationale for Combination Approaches in Patients with EGFR-Expressing Solid Tumors

Poster 112

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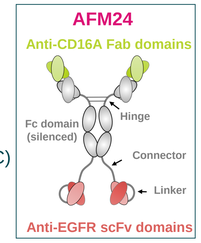
BACKGROUND

- Epidermal growth factor receptor (EGFR) is frequently overexpressed on the cell surface in solid tumors, and is associated with poor prognosis[1]
- Some patients do not respond to EGFR inhibitors[2], and in patients that do, acquired resistance invariably occurs[3]; novel therapies acting independently of EGFR signaling are required
- AFM24 is a bispecific Innate Cell Engager (ICE®) derived from the Redirected Optimized Cell Killing (ROCK®) antibody platform
- AFM24 is a tetravalent EGFR/CD16A-specific IgG1-scFv fusion antibody (scFv-IgAb) with a silenced IgG1 Fc
- AFM24 engages CD16A on natural killer (NK) cells and macrophages with a higher affinity than monoclonal antibodies, and triggers antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), respectively, directed at EGFR-expressing (EGFR$^+$) cancer cells[4]
- Preclinical data have shown that AFM24 can induce NK cell-mediated killing of EGFR$^+$ solid tumor cell lines, independent of EGFR mutational status[5]
- An ongoing phase 1/2a study (NCT04259450) is seeking to establish the safety and efficacy of AFM24 monotherapy in EGFR$^+$ solid tumors



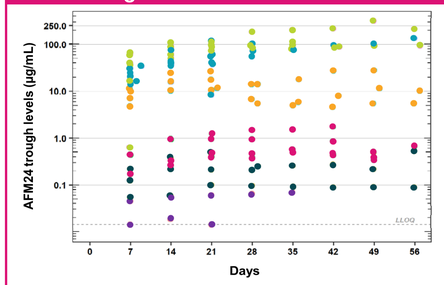
OBJECTIVE

Assess the exposure and pharmacodynamic effects of AFM24 in patients with EGFR$^+$ tumors using correlative science

RESULTS

- As of 29th October 2021, a total of 29 patients have been treated with AFM24 across six dose levels (14–480 mg)
  - The median (range) number of AFM24 doses administered was 8 (1–29)
  - Best objective response was stable disease in 8 of 24 response-evaluable patients (at least one post-baseline computed tomography scan available ≥6 weeks post-initiation of therapy)

Dose-proportional PK was observed between 320 mg and 480 mg



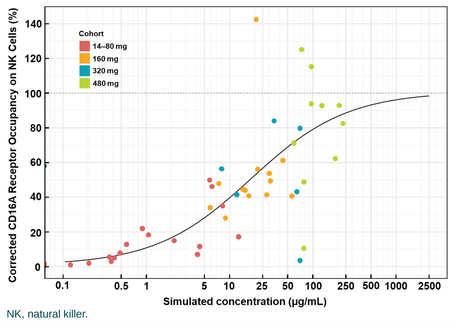
Steady state (C_{trough},ss) was achieved between 21–28 days at 1×/week dosing.

Apparent half-life is estimated to be 11.2 days at doses ≥320 mg.

The RP2D is 480 mg

PK, pharmacokinetics; RP2D, recommended Phase 2 dose.

Peripheral CD16A receptor occupancy by AFM24 levels off at concentrations above 320 mg



Exposure-response relationship of CD16A receptor occupancy with AFM24 levels in circulating blood.

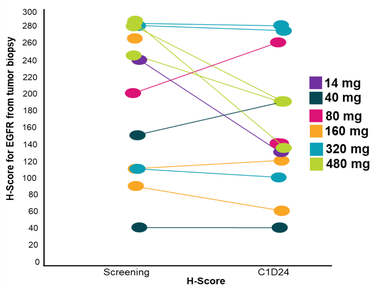
Receptor occupancy approaches saturation of CD16A with AFM24 on NK cells at 480 mg.

CD16A receptor occupancy levels expected in the tumor are above threshold receptor occupancy needed for maximal efficacy as shown *in vitro*.

Log transformed x-axis.

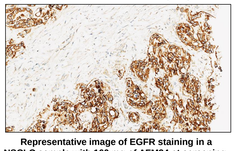
NK, natural killer.

Tumor EGFR expression was maintained during AFM24 treatment



Tumor biopsies were taken at baseline and on Day 24 of treatment. Specimens were stained by IHC and scored with the H-Score.

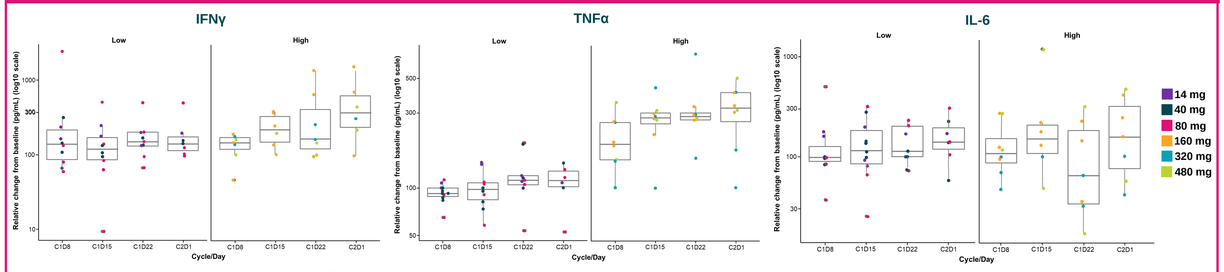
The H-Score calculates a score from 0 to 300 based on the percentage of cells stained at different intensities viewed at various magnifications. All analyzed samples were positive for EGFR at varying levels.



Representative image of EGFR staining in a NSCLC sample with 160 mg of AFM24 at screening.

EGFR, epidermal growth factor receptor; IHC, immunohistochemistry.

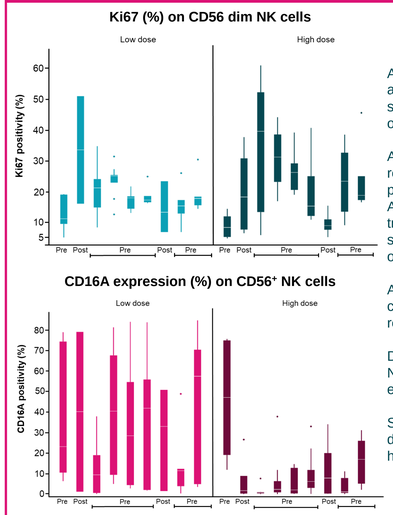
Increases in pro-inflammatory cytokines at higher doses of AFM24 may reflect sustained activation of immune cells



Serum samples were taken one week after each AFM24 dose and just prior to the next dose. Measurements at Cycle 1, Day 1 (C1D1) were considered as baseline. Samples were grouped by AFM24 dose level: low (14–80 mg) versus high (160–480 mg). AFM24 at ≥160 mg showed an increase in IFNγ and TNFα, indicating activation of immune cells. Levels in the pg range suggest that these cytokines are not being produced in high amounts by blood lymphocytes, thus are not indicative of CRS, but may rather occur as a result of tissue effects. IL-6 was not upregulated, further indicating a good safety profile of AFM24. Log transformed y-axis.

CRS, cytokine release syndrome; IFNγ, interferon-γ; IL-6, interleukin 6; TNFα, tumor necrosis factor-α.

Peripheral blood NK cells are activated by AFM24



Activation of NK cells occurs even at low doses (14–80 mg) as shown by acute upregulation of Ki67.

At higher doses (160–480 mg), a reduction in CD16A$^+$ NK cells in peripheral blood is observed. Activated NK cells may shed or transiently down regulate CD16A, so reduced CD16A is also a sign of activation.

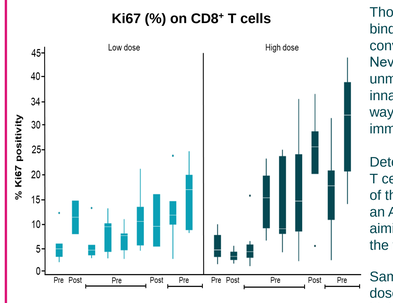
Additionally, we hypothesize that circulating activated NK cells are retained in EGFR$^+$ (tumor) tissue.

Death of CD16A$^+$ NK cells due to NK fratricide by AFM24 could be excluded experimentally *in vitro*.

Samples were grouped by AFM24 dose level: low (14–80 mg) versus high (160–480 mg).

Pre, pre-dose; post, post-dose; NK, natural killer.

In peripheral blood, CD8$^+$ cytotoxic T cells become activated as an indirect effect of AFM24



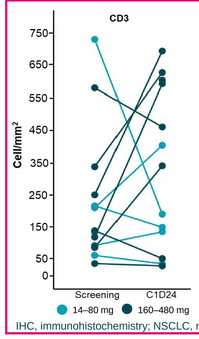
Though AFM24 does not directly bind to adaptive immune cells, it conveys an indirect effect on T cells. Nevertheless, as in "normal", unmodified immune responses, the innate immune system paves the way for an effective adaptive immunity against a common enemy.

Detecting activation of cytotoxic T cells subsequent to the activation of the innate immune cells points to an AFM24 induced indirect effect aiming for a concerted destruction of the tumor.

Samples were grouped by AFM24 dose level: low (14–80 mg) versus high (160–480 mg).

Pre, pre-dose; post, post-dose.

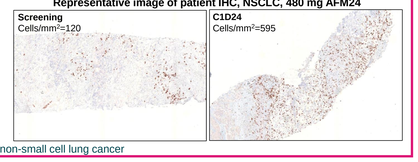
Biopsy analysis shows an increase in T cells within the tumor



Fine needle biopsies of patients treated with AFM24 were taken at screening (before treatment) and approximately 3 weeks into treatment (Cycle 1, Day 24, 48 h after the 3rd treatment – biopsies are variable and reflect only a snapshot of analyzed patient samples).

An increased number of T cells, detected using CD3 as a T cell marker in IHC, in tumour biopsies of patients treated with AFM24 suggests an increase in inflammation and activation of the adaptive immune system.

Representative image of patient IHC, NSCLC, 480 mg AFM24

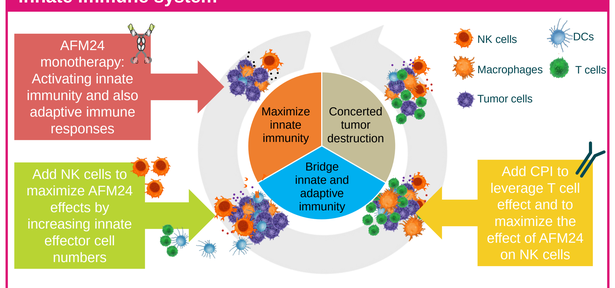


IHC, immunohistochemistry; NSCLC, non-small cell lung cancer

CONCLUSIONS

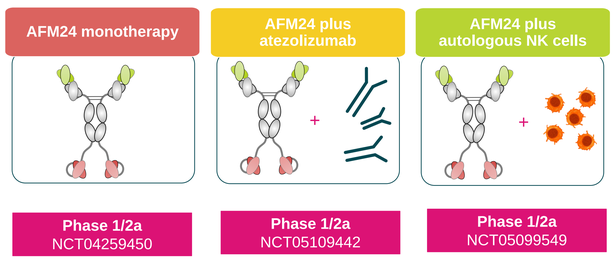
- **In line with the mechanism of action of AFM24, longitudinal effects on cells of the innate immune system were confirmed**
- **An additional activation of cytotoxic T cells in the periphery and infiltration of T cells into the tumor bed was revealed, suggesting stimulation of anti-cancer immunity beyond the innate immune system**
- **This supports the rationale for AFM24 monotherapy and for the combination approaches that are currently being investigated:**
  - **AFM24 combined with autologous NK cell therapy (NCT05099549)**
  - **AFM24 combined with immune checkpoint inhibition (NCT05109442)**

Every successful immune response begins with the power of the innate immune system



CPI, checkpoint inhibitors; DC, dendritic cells; NK, natural killer.

AFM24 development strategy



Atezolizumab is an immune checkpoint inhibitor (CPI). NK, natural killer.

AFM24 monotherapy: Phase 1 study design

PHASE 1: DOSE ESCALATION ONGOING

R/R patients with EGFR$^+$ tumors (N=29)

- All advanced/metastatic solid malignancies known to express EGFR
- Majority of the cohort had CRC (N=16) or NSCLC (N=7)
- R/R to ≥1 anti-cancer therapy, with documented progression during or following treatment
- **Primary endpoint:** Dose-limiting toxicities
- **Secondary endpoints:** Safety, preliminary efficacy, duration of response, pharmacokinetics and immunogenicity

Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6
14 mg N=2	40 mg N=6	80 mg N=4	160 mg N=5	320 mg N=6	480 mg N=6

CRC, colorectal cancer; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; R/R, relapsed/refractory

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